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Presentation of the Self Antigen Myelin Basic Protein by Dendritic Cells Leads to Experimental Autoimmune Encephalomyelitis

Bonnie N. Dittel, Irene Visintin, Raina M. Merchant, and Charles A. Janeway, Jr.

Bone marrow (BM)-derived dendritic cells (DC) are potent stimulators of naive CD4+ T cell activation. Because DC are efficient at Ag processing and could potentially present self Ags, we investigated the role of DC in the presentation of an encephalitogenic peptide from myelin basic protein (Ac1–11) in the induction of experimental autoimmune encephalomyelitis (EAE). To determine if DC could prime for EAE, we transferred DC pulsed with Ac1–11 or with medium alone into irradiated mice in combination with CD4+ T cells isolated from a mouse transgenic for a TCR specific for Ac1–11 + I-Aa. Mice transferred with Ac1–11-pulsed DC developed EAE 7–10 days later, whereas mice receiving medium-pulsed DC did not. By day 15, all mice given peptide-loaded DC had signs of tail and hind limb paralysis, and by day 20 infiltration of Ac1–11-specific CD4 T cells was detected in the brain parenchyma. We also demonstrated interactions between Ac1–11-pulsed DC and Ac1–11-specific T cells in the lymph nodes 24 h following adoptive transfer of both cell populations. These data show that DC can efficiently present the self Ag myelin basic protein Ac1–11 to Ag-specific T cells in the periphery of mice to induce EAE. The Journal of Immunology, 1999, 163: 32–39.

Dendritic cells (DC) are specialized APC that possess the capacity to activate naive T cells (1, 2). DC residing in the T cell areas of lymph nodes are of bone marrow (BM) origin (3) and have been shown to migrate from the peripheral blood into the spleen and to effenter lymphatics into the lymph nodes following activation, presumably after encountering Ag (4, 5). Once established in the lymph node, DC characteristically express MHC class II molecules and many of the adhesion molecules (ICAM-1, LFA-1, and LFA-2) involved in interaction with naive T cells and the costimulatory molecules (B7-1, B7-2, heat stable Ag, and CD40) needed for naive T cell priming (1, 6). In particular, MHC class II and B7-2 are constitutively expressed at very high levels (1, 6, 7). The morphology of DC facilitates interaction with T cells by means of thin cytoplasmic sheets, also referred to as veils, extending as processes into the surrounding lymph node (1). Visualization of interactions between DC and T cells in the T cell-rich areas of lymph nodes have been demonstrated for T cells in response to a specific peptide Ag (8).

It has been hypothesized that T cell-mediated autoimmune diseases are the result of inappropriate Ag presentation of either a self-Ag or an Ag with the capacity to mimic a self-Ag in the peripheral lymphoid tissues. An activated or memory T cell specific for a self peptide then has the potential to meet the self Ag where it is naturally expressed. In the case of experimental autoimmune encephalomyelitis (EAE), autoreactive T cells would reencounter their specific self-Ag in the CNS. Immunization of mice with several different protein components of myelin, including myelin basic protein (MBP), proteolipid protein (PLP), proteolipid glycoprotein, and myelin oligodendrocyte glycoprotein, are capable of eliciting an immune response resulting in the quintessential symptoms of EAE: ascending paralysis involving the tail and then the limbs (9, 10). EAE is a demyelinating disease in rodents and is used as a model of the human disease multiple sclerosis (9–11). EAE is characterized by focal areas of inflammation and demyelination throughout the CNS and can be actively induced in genetically susceptible animals by the injection of myelin Ags in CFA or passively by the adoptive transfer of activated CD4 T cells producing type 1 cytokines specific for myelin Ags, provided they express the adhesion molecule VLA-4 (9, 10, 12). In I-Ab mice, the primary immunogenic peptide of MBP is the acetylated N-terminal peptide from residues 1 to 11 (Ac1–11) (9).

In this study, we show that DC capable of processing and presenting the self Ag MBP migrate to the lymph nodes via the effenter lymphatics, and interact with naive Ac1–11-specific T cells leading to the induction of EAE. In addition, Ac1–11-specific T cells were observed in the brains and spinal cords of mice 20 days following this unique means of EAE induction. These data demonstrate that presentation of a self Ag by DC in the periphery results in the activation of MBP-specific T cells and can lead to an overt autoimmune disease.

Materials and Methods

Mice

B10.PL mice (I-Ab) were purchased from The Jackson Laboratory (Bar Harbor, ME). MBP-TCR transgenic (tg) mice were generated as previously described (13) and reared in our colony at Yale University. All mice used were between 5 and 10 wk of age.

Peptides and Abs

The MBP Ac1–11 (Ac-ASQKRPSQSK) and the MBP21–140 (GFGYG GRASDYKSAHKFGK) peptide were synthesized and HPLC purified by...
the W. M. Keck Biotechnology Resource Laboratory (Yale University). GK1.5 (rat anti-mouse CD4), HO-2.2 (mouse anti-mouse CD8), and RA3-3A1/6.1 (rat anti-mouse B220) were purchased from the American Type Culture Collection (Manassas, VA) and maintained in our laboratory. A clonotypic mAb (19G) specific for the MBP-TCR (12), Y3JP (mouse anti-mouse I-A), and Y19 (rat anti-mouse Thy1) were produced in our laboratory using standard hybridoma generation techniques. Anti-mouse CD4-biotin and anti-mouse Vβ1.8.2-biotin were purchased from PharMingen (San Diego, CA).

Cells

DC were isolated as previously described (14). Briefly, BM was flushed from the femurs and tibias of B10.PL mice. BM cells were depleted of B cells, T cells, and I-A+ cells using Abs and complement. The mAb used were GK1.5, HO-2.2, Y19, RA3-3A1/6.1, and Y3JP. The remaining mononuclear cells were plated in 12-well plates at 0.5 × 10^6 cells/ml in RPMI 1640 medium supplemented with 5% FCS, 1 mM L-glutamine, 20 μg/ml gentamicin, 50 μM 2 ME, and 1% culture supernatant from 35SLP cells transfected with a GM-CSF construct (kindly provided by Fritz Melchers, Basel Institute of Immunology, Basel, Switzerland). After 2 days, the nonadherent cells were gently removed and discarded, and fresh medium was added every 2 days to the remaining adherent cells. On day 6, the nonadherent and loosely adherent cells were removed and replated into 100-mm culture tissues in the above medium. Following overnight incubation, the nonadherent cells were collected and pulsed with medium alone or Ac1–11 (100 μg/ml) for up to 6 h in 100-mm culture dishes and washed twice in PBS before transfer into mice. Purity of the T cells was > 60% as determined by flow cytometry examining expression of B7-2 and high levels of MHC class II (Y3JP). MBP-TCR CD4 T cells were isolated from the spleen of MBP TCR tg 6–8-week-old B6.PL mice. Briefly, spleens were minced and RBC were removed following depletion by depletion of cells expressing CD8, CD20, and I-A using mAb and complement. The mAb used were HO-2.2, RA3-3A1/6.1, and Y3JP. Purity of the T cells was assessed using mAb specific for mouse CD4, CD8, B220, αβ TCR, Vβ8.1.8.2, and the MBP-TCR clonotypic mAb 19G. The MBP-TCR 31 clone was isolated from the spleen of a MBP tg mouse we prepared (13) that expresses a TCR specific for Ac1–11 and restricted to αβ TCR and expresses the MBP-TCR as assessed by staining with mAb 19G.

To examine the presentation of a MBP self peptide by DC, we used a CD4+ T cell clone (MBP-TCR 31) isolated from a mouse tg for the rearranged TCR α- and β-chains of a cloned T cell line called clone 19 that is restricted to I-Aα and specific for Ac1–11 (12). As shown in Fig. 1A, proliferation of the MBP-TCR 31 clone occurred following presentation of the Ac1–11 peptide by BM-derived DC in a dose-dependent manner. The specificity of the MBP-TCR 31 clone to Ac1–11 is demonstrated by the lack of proliferation to the MBP peptide composed of residues 121–140, which binds to I-Aα and is encephalitogenic in (B10.PL × SJL)F1 mice (B. N. Dittel and C. A. Janeway, unpublished observations). Because the goal of this study was to use Ac1–11-pulsed DC in vivo to generate an immune response culminating in EAE, we examined the proliferative response of the MBP-TCR 31 clone to Ac1–11-pulsed DC. As shown in Fig. 1B, proliferation of the MBP-TCR 31 clone was enhanced with the addition of increasing numbers of Ac1–11-pulsed DC. In contrast, no proliferation was observed when DC were pulsed with medium alone. Because Ag uptake and processing have been shown to change with DC maturation (17), we tested whether the DC used in this study were capable of protein Ag uptake leading to processing and presentation of the Ac1–11 peptide. This is demonstrated in Fig. 1C by the proliferative response of the MBP-TCR 31 DC with whole mMBP.

Direct interaction between Ac1–11-pulsed DC and MBP-TCR tg T cells is observed in the draining lymph node

DC have been shown to form clusters with Ag-specific T cells in vitro (18) and in vivo (8). Because this clustering is believed to

EAE induction

Irradiated (600 rads) female B10.PL mice were s.c. injected in the footpads (0.5 × 10^6 cells) and in each internal flank (1 × 10^6 cells) with BM-derived DC in PBS that had been pulsed with medium alone or Ac1–11. Twenty-four hours later, 10 × 10^6 MBP-TCR CD4 splenic T cells in PBS, isolated as described above, were injected i.v. into each animal. Individual animals were assessed daily starting at day 6 for symptoms of EAE and scored using a scale from 1 to 5 as follows: 0, no disease; 1, limp tail and/or wobbly walk; 2, hind limb paresis; 3, hind limb paralysis; 4, hind and fore limb paralysis; 5, death.

Labeling and in vivo detection of T cells and DC

DC and MBP-TCR tg T cells isolated as described above were labeled with the fluorescent dyes 3,3′-dioctadecylxocarbocyanine perchlorate (DHO) (6 ng/ml) and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiD, 2.5 mg/ml) (Molecular Probes, Eugene, OR), respectively, for 30 min at 37°C after which they were washed three times in PBS. DC (0.5 × 10^6) pulsed with medium alone or with 100 μg/ml Ac1–11 were injected s.c. into each footpad followed by i.v. injection of 10 × 10^6 MBP-TCR tg CD4 T cells. After 24 h, the popliteal lymph nodes were harvested and fixed in paraformaldehyde-lysine-periodate overnight. The lymph nodes were sucrose infused prior to embedding in Tissue Tek OCT (Miles, Elkhart, IL) and frozen in isopentane. Frozen sections 7 μm thick were generated and visualized on a fluorescent microscope.

Immunohistopathological analysis

Brains and spinal cords were removed from perfused mice and fixed in paraformaldehyde-lysine-periodate. After 24 h, the tissues were sucrose infused, embedded in Tissue Tek OCT (Miles), and frozen in isopentane. For immunohistochemistry, frozen sections 7 μm thick were stained with biotinylated anti-CD4, biotinylated anti-Vβ8.1.8.2, or biotinylated 19G. The color was developed using HistoMark Red Phosphatase System (Kiregaard & Perry Laboratories, Gaithersburg, MD) and counterstained with hematoxylin.

Results

BM-derived DC present a MBP self peptide to a MBP-specific T cell clone

To examine the presentation of a MBP self peptide by DC, we derived DC from the BM of the B10.PL mice (H-2b), a mouse strain that is genetically susceptible to the CNS autoimmune disease EAE. To detect Ag presentation by the DC, we used a CD4+ T cell clone (MBP-TCR 31) isolated from a mouse tg for the rearranged TCR α- and β-chains of a MBP-TCR clone as previously described (15) using a modified procedure of Srendi et al (16). The MBP-TCR 31 clone was maintained by restimulation in Click’s Eagle Hank’s amino acid (EHAA) medium containing 10% FCS, 2 U/ml IL-2, and 5 μg/ml Ac1–11 in the presence of inactivated spleen cells from B10.PL mice (I-Aα) every 3–6 wk and allowed to rest at least 10 days before use. The MBP-TCR 31 clone is CD4+ and expresses the MBP-TCR as assessed by staining with mAb 19G.

T cell activation assay

MBP-TCR 31 cloned cells (1 × 10^4) or splenic CD4+ MBP-TCR CD4 T cells (1 × 10^4) were incubated with 1 × 10^6 BM-derived DC isolated as described above in the presence or absence of 1:10 dilutions of the peptides Ac1–11, or MBP121–140, from 0.001 to 1 μg/ml in Click’s EHAA medium containing 5% FCS. In addition, 1 × 10^5 cloned T cells were incubated with increasing numbers of DC that had been pulsed with medium alone, 100 μg/ml Ac1–11, or 100 μg/ml whole mouse MBP (mMBP). CD4+ T cells (2 × 10^5), isolated from the spleen of a mouse 30 days posttransfer of Ac1–11-pulsed DC and MBP-TCR CD4 T cells (as described below), were cocultured with 2 × 10^5 inactivated H-2d splenocytes in the presence of increasing concentrations of Ac1–11. Proliferation was detected at 72 h by the addition of 1 μCi [3H]Thymidine to each well for the last 18–19 h of culture. Individual data points were set up in duplicate.

Cytokine secretion

IL-2 production from splenic CD4+ MBP-TCR CD4 T cells was detected using CTL2-2 responder cells. Culture supernatants were collected 24 h following coculture of 1 × 10^6 MBP-TCR CD4 T cells and 1 × 10^5 inactivated H-2d splenocytes in the presence of increasing concentrations of Ac1–11. Prior to the addition of 5000 CTL2-2 cells, the culture supernatants were frozen at −70°C to kill any viable cells. Following culture for 18 h, the cells were pulsed with 1 μCi [3H]Thymidine and harvested after 4 h. INF-γ was detected from culture supernatants collected 24 h following coculture of 2 × 10^6 CD4+ splenic T cells, isolated from a mouse injected 30 days prior with Ac1–11-pulsed DC and splenic MBP-TCR and T cells, and 2 × 10^5 inactivated H-2d splenocytes in the presence of increasing concentrations of Ac1–11. INF-γ was detected by ELISA as previously described (15).

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alone (and mice were cocultured with the Ac 1–11 -specific MBP.TCR 31 clone (10,000 the MBP-TCR. A clusters of cells composed of green fluorescing DiO-labeled DC; cells/well) in the presence of 1 labeled T cells and the DiO-labeled DC. When DC pulsed with B power magnification field is shown in Fig. 2 with the self Ag Ac1–11 as compared to DC pulsed with medium naive CD4 T cells in the lymph node of B10.PL mice when pulsed erentially form interactions and clusters with MBP-TCR bearing facilitate Ag presentation, we examined whether DC would pref- m clusters with MBP-TCR bearing CD4 T cells and tested for proliferation and cytokine production. The successful priming of MBP-TCR T cells is shown in Fig. 3b by the dose-dependent proliferation and IL-2 production following stimulation with Ac1–11. Because we detected a small population of memory cells that are known to produce abundant quantities of either Th1 or Th2 cytokines upon stimulation (19), we examined the MBP-TCR CD4 T cells for the production of IL-4 and IFN-γ (Fig. 3A). We did not detect either IL-4 or IFN-γ by ELISA (data not shown). The production of IL-2 in the absence of IL-4 and IFN-γ further suggests a naive phenoty- pe of the tg T cells. To examine whether we could prime for a Th1 or Th2 response using DC, we isolated CD4+ splenocytes from mice 30 days following transfer of Ac1–11-pulsed DC and MBP-TCR CD4 T cells and tested for proliferation and cytokine production. The successful priming of MBP-TCR T cells is shown in Fig. 3b by the dose-dependent proliferation and IFN-γ production in response to stimulation with Ac1–11. No IL-4 production was detected (data not shown), demonstrating priming of a Th1, but not Th2, response.

Interactions between Ac1–11-pulsed DC and MBP-TCR CD4 T cells in vivo results in EAE

Because we were able to demonstrate an Ag-specific interaction between DC and MBP-TCR CD4 T cells in vivo in lymph nodes leading to a Th1 response, we asked whether this interaction could lead to the induction of EAE. As shown in Fig. 4A, symptoms of EAE occurred as early as day 8 following adoptive transfer of naive MBP-TCR CD4 T cells and Ac1–11-pulsed DC. As a comparison, animals that were transferred with MBP-TCR CD4 T cells and medium-pulsed DC exhibited no signs of EAE (Fig. 4A). All animals that received peptide-loaded DC exhibited signs of EAE by day 11, and peak disease was reached on day 20. The animals were sacrificed on day 20 at the peak of disease for the analysis of CNS cell infiltration (Fig. 5). In an extended experiment in which the animals continued being examined for EAE after the peak day of disease, the mice were able to resolve most symptoms of disease and recover (Fig. 4B). These data show that DC can present the Ac1–11 self peptide to MBP-TCR T cells resulting in autoimmune- nity. The induction of EAE is dependent upon the transfer of live DC, as i.p. injection of Ac1–11 (100 µg) or transfer of paraformaldehyde-fixed DC pulsed with Ac1–11 in combination with trans- ferred MBP-TCR T cells did not result in EAE. In the experiments shown in Fig. 4, the DC were injected 24 h prior to the adoptive transfer of MBP-TCR T cells. This time delay is not required, as EAE also occurs when the DC and T cells are transferred on the same day (data not shown).
MBP-TCR CD4 T cells are detected in the CNS of animals with EAE

Because EAE is a disease of the CNS associated with the destruction of surrounding oligodendrocytes of the myelin sheath, disease symptoms should be accompanied by infiltration of encephalitogenic T cells into the brain and spinal cord. Using the animals shown in Fig. 4, we examined brain and spinal cord for the presence of MBP-TCR CD4 T cells. Because the MBP-TCR contains a Vβ8.2β-chain, we used a mAb specific for Vβ8.1/8.2 to detect the presence of transgenic T cells. We were able to visualize the presence of both CD4+ (Fig. 5A) and Vβ8.2+ (Fig. 5B) cells in the brains and spinal cords (data not shown) of mice with active EAE. To confirm that the T cell infiltration observed contained MBP-TCR CD4 T cells, we stained the tissue sections with a clonotypic mAb specific for the MBP-TCR (Fig. 5C). Mice from the same experiment which received medium-pulsed DC and did not exhibit signs of EAE showed little to no detectable infiltration of CD4+, Vβ8.2+, or MBP-TCR+ (Fig. 5D, E, and F, respectively) cells in the brain. We have previously shown that in vitro-activated Ac1–11-specific cloned T cells induced EAE upon adoptive transfer and

**FIGURE 2.** DC pulsed with Ac1–11 interact with MBP-TCR naive CD4 T cells in the lymph nodes, whereas medium-pulsed DC do not. BM-derived DC pulsed with medium alone or with Ac1–11 were injected into the footpads of B10.PL mice along with the i.v. injection of naive MBP-TCR CD4 splenocytes. Prior to injection, the DC were labeled with the fluorescent dye DiO (green) and the T cells were labeled with Dil (red). After 24 h, the lymph nodes were harvested, and frozen sections were examined for interactions between DC and T cells. A and B show low power magnification, and C and D show high power magnification. Areas of direct interaction between Ac1–11-pulsed DC are seen by the yellow fluorescence in B and by direct cell contacts in D. A and C are from mice that received DC pulsed with medium alone.

**FIGURE 3.** Splenic MBP-TCR tg T cells have a naive phenotype and can be primed in vivo with Ac1–11-pulsed DC to become Th1 T cells. A, CD4+ T cells isolated from the spleen of a MBP-TCR tg mouse were cocultured with B10.PL splenocytes (H-2k) in the presence 1:10 dilutions of Ac1–11 from 10 to 0.001 μg/ml. B, CD4+ T cells isolated from the spleen of a mouse 30 days following injection of Ac1–11-pulsed DC and MBP-TCR CD4 T cells were cocultured with B10.PL splenocytes (H-2k) in the presence 1:4 dilutions of Ac1–11 from 40 to 0.16 μg/ml. Proliferation (A and B) and subsequent secretion of IL-2 (A) or IFN-γ (B) within the same culture are shown. Proliferation was measured by 3H]Tdr incorporation and presented as CPM on the left y-axis (A and B). Secretion of IL-2 was assayed using CTLL cells, as described in Materials and Methods, and shown on the right y-axis in A (○) as U/ml. IFN-γ was assayed by ELISA, as described in Materials and Methods, and shown on the right y-axis in B (□) as pg/ml. All individual data points were performed in duplicate and averaged.
are detected in the CNS of sick animals (12). Our ability to reproduce this result using our DC induction model is important for future studies on the dynamics of cell migration into the brain and on demyelination.

Discussion

EAE, an animal model of the human CNS autoimmune disease multiple sclerosis, can be induced actively or passively in genetically susceptible rodent strains. In mice, active induction of EAE requires immunization with either protein or peptide myelin Ags emulsified in CFA in combination with pertussis toxin injections. After 2 wk the first signs of disease, a limp tail and hind limb weakness, are observed. Depending on the mouse strain, the disease is either an acute monophasic disease course as observed in B10.PL mice (20) or relapsing and remitting as observed in SJL/J mice (21, 22). Passive induction of EAE requires the presolation of CD4+ T cells, generally of the Th1 phenotype, with Ag specificity for myelin Ags. Following in vitro activation, the T cells are adoptively transferred into irradiated recipient mice with disease symptoms occurring as early as 6–7 days later with transfer of large numbers of encephalitogenic T cells (12). The disease course is variable and can be acute or chronic monophasic or relapsing and remitting and is dependent on the number of T cells transferred and the mouse strain used in the study. Both models of EAE induction have been used extensively, with the active model most useful for studying the parameters involved in the initiation, effector, and recovery phases in one disease process.

FIGURE 4. Interactions between Ac1–11-pulsed DC and MBP-TCR CD4 naive T cells in vitro leads to EAE. BM-derived DC were pulsed in vitro with medium alone or 100 μg/ml Ac1–11 and adoptively transferred into irradiated B10.PL mice. Twenty-four hours later, naive splenic CD4 T cells isolated from MBP-TCR tg mice were adoptively transferred into the same animals. Animals were assessed daily for clinical signs of EAE. Animals were scored on a scale of 1–5 as described in Materials and Methods. Animals receiving DC pulsed with Ac1–11 are shown as filled circles, and animals receiving medium-pulsed DC are shown as open circles. Data shown in A and in B are from two separate experiments. Each group represents the average disease score of five mice, with the SE given.

In this study, we describe a novel method of EAE induction using BM-derived DC pulsed with the Ac1–11 MBP peptide capable of interacting with and activating naive Ac1–11-specific T cells in vivo leading to EAE. We sought to develop a single model of EAE facilitating the study of the autoimmune process including initiation, effector, and recovery phases in one disease process. Building upon our previous work using B10.PL mice (20) and utilizing our Ac1–11-specific TCR tg mouse (13), we have successfully used DC as adjuvants to induce EAE. In our model, BM-derived DC were pulsed with Ac1–11 prior to i.v. injection into irradiated recipient mice, followed by i.v. injection of splenic naive CD4+ T cells. In concordance with our previous studies using B10.PL mice (20), we observed an acute monophasic disease course (Fig. 4B) followed by infiltration of MBP-TCR tg T cells into the brain parenchyma at a level we have previously observed only in passive EAE induction (Fig. 5) (12).

One of the major drawbacks to the study of EAE using active induction models is the necessity to use CFA as an adjuvant. CFA has been shown to preferentially induce Th1 immune responses over Th2 responses (23). This immune skewing can be beneficial, since a variety of studies have shown that the pathogenic T cell is typically of the Th1 phenotype (10), but has limitations in determining a role for Th2 cells. Th2 cells have been shown to be encephalitogenic under specific conditions (24) and are thought to be important in protection from EAE (10, 25, 26). Thus, the absence of CFA in our EAE model will allow a more precise study of the dynamics of cytokine profiles in EAE that have been suggested to switch from a Th1 pattern during the effector phase to a Th2 pattern during the recovery phase (10). In addition, we have eliminated the need for pertussis toxin, which inhibits G-protein function and has been shown to be immunomodulatory (27, 28).

In the mouse, the induction of EAE following the adoptive transfer of activated encephalitogenic T cells typically requires sublethal irradiation of the host animal. The irradiation is believed to facilitate the migration of T cells into the CNS, perhaps by up-regulating cytokines and adhesion molecules, leading to disease. Although irradiated animals were used in the experiments in Fig. 4, we were able to induce EAE in nonirradiated mice using our DC-based model of EAE induction (data not shown). Thus, DC alone in the absence of a stimulus provided by the mycobacteria in CFA, and in the absence of inflammatory signals provided by irradiation, can present self Ags resulting in the activation of self-reactive T cells leading to autoimmunity.

DC pulsed with tumor Ags have been used successfully in the treatment of a variety of animal tumor models (29). Although these studies have confirmed the efficient immune stimulatory activity of the adaptive immune response by DC, there remains much to learn about DC cell physiology. DC of BM origin are now known to reside in most tissues of the body. These DC are thought to be immature with the capacity for efficient and rapid Ag uptake leading to processing and presentation of Ags. In the case of foreign microbial Ags that activate DC, the DC are triggered to migrate to the draining lymph nodes via the afferent lymphatics. DC activation also initiates a rapid maturation process culminating with a mature DC with limited ability to take up and process Ag, high cell surface levels of MHC class II and B7-2, and a striking change in
phenotype to a cell with numerous extended processes. This mature DC, now residing in the T cell zone, has a very slow turnover of peptide-bound MHC class II molecules on the cell surface (30). This DC maturation process was dramatically illustrated in vitro using BM-derived DC by tracking the location of MHC class II molecules from intracellular vesicles to the cell surface in combination with changes in morphology (17). Thus, the use of DC therapeutically requires the DC to be immature upon Ag pulsing. This is particularly important with protein Ags where peptide exchange on the cell surface is not a feasible mechanism (31). Although DC at various stages of maturation can present peptide Ags to T cell clones (32), only immature and not fully mature DC were shown to stimulate T cell clones when whole protein Ag was used (18). In this study, we used BM-derived DC that were enriched by replating overnight to allow cells of the monocyte lineage to strongly adhere. Although replating induces DC maturation, proliferation of the Ac1–11-specific MBP.TCR 31 clone occurred when the stimulating Ag was whole mMBP protein (Fig. 1C). This result demonstrates that the DC used in this study were capable of protein Ag uptake leading to processing and presentation.

Our use of T cells that are activated in vivo on peptide-loaded DC more closely mimics the actual series of events that occur between T cell priming and EAE induction in vivo, as compared with peptide immunization or the use of in vitro-activated T cell clones. In previous experiments, we have successfully induced EAE using a T cell clone expressing the Ac1–11-specific TCR from which the MBP tg mouse was generated (12). Although these experiments were successful, the use of T cell clones has limitations. The most problematic is the loss of encephalitogenic potential with continuous passage in culture as the result of phenotypic changes. Although phenotypic variations is not a concern in our model, a potential drawback to the use of CD4+ T cells from the MBP-TCR tg mouse is the presence of Ac1–11-specific Th1 memory cells that could rapidly expand and produce Th1 cytokines leading to EAE. In our model, this seems not to be the case since ~95% of the CD4+ T cells bear the Vβ8.2 TCR chain with only 1.2–1.5% of the starting tg T cell population of the memory phenotype (data not shown). This is consistent with a report by Linton, et al. (33), showing that CD4+ tg T cells in the AND TCR tg mouse are of a naive phenotype, even upon aging. In contrast, the memory cells detected showed little to no expression of the TCR tg. In addition, we have been able to induce severe EAE with hind limb paralysis using Ac1–11-pulsed DC and transfer of only 4 x 10^6 Vβ8.2+ MBP-TCR tg T cells, of which only 6 x 10^4 would be memory cells (data not shown). Although EAE has been shown to be induced following transfer of 1 x 10^5 activated encephalitogenic T cells, EAE onset was delayed until approximately day 28 and required both irradiation and injection of Bordetella pertussis (34). In addition, we have been able to induce EAE following the elimination of memory cells by cell sorting (data not shown).

A further expansion of our DC-based EAE induction model would be to induce EAE with Ac1–11-pulsed DC in the absence of transferred MBP-TCR T cells. To date, we have not been successful in inducing EAE in B10.PL mice without the transfer of MBP-TCR tg T cells (data not shown). We feel that a low precursor frequency of autoreactive T cells in the B10.PL mouse is the reason for the absence of EAE induction.

FIGURE 5. Infiltration of MBP-TCR CD4 cells is observed in the brain parenchyma in a perivascular pattern in animals with EAE induced with Ac1–11-pulsed DC. The brains from animals shown in Fig. 4A were harvested on day 20 following DC transfer and cryopreserved. Frozen sections of brain cut coronally were obtained and stained with Abs specific for CD4 (A and D), Vβ8.2 (B and E), and with a clonotypic mAb (19G) with specificity for the MBP-TCR (C and F). A–C show brain tissue from one representative mouse that had clinical signs of EAE. D–F show brain tissue from one representative mouse that received medium-pulsed DC and did not show clinical signs of disease. The cells colored reddish/pink are cells staining positive for CD4, Vβ8.2, or 19G.
I-Aα MHC class II molecule (35) is not sufficient for the development of an immune response leading to autoimmunity. Another contributing factor is the transient presence of the peptide-pulsed DC in the lymph nodes, which decline in number 24 h following transfer (8). The use of CFA allows the slow constant release of Ag over a time frame of weeks. We feel that the transferred DC, although competent APC able to activate naive T cells, do not remain in the lymph nodes for a sufficient time to induce an autoimmune response. We are currently performing experiments using a modified Ac1–11 peptide with increased affinity for the I-Aα MHC class II molecule combined with twice weekly transfers of DC to test our hypothesis.

A common strategy employed by a variety of investigators for preventing or diminishing the clinical symptoms of EAE is tolerance induction. Various mechanisms of tolerance induction have been utilized including the use of peptides (36, 37), thymic dendritic cells (38), and blocking of costimulatory molecules (39, 40).

A likely common mechanism of these tolerogenic models is the prevention of the primary immune response. However, in multiple sclerosis patients, T cells with specificity for myelin Ags existing in the peripheral blood have an activated phenotype when compared to T cells from normal controls with comparable specificities (41–43). The long-term persistence of MBP-specific T cell clones in a multiple sclerosis patient suggests in vivo activation of the self reactive T cells (44). Thus, a mechanism of tolerance induction capable of rendering the myelin Ag-specific T cells unresponsive would be beneficial. We are currently using our DC EAE induction model to examine whether DC can deliver a tolerogenic signal to Ac1–11-specific naive T cells present prior to primary immunization and, more importantly, to primed CD4+ T cells after immunization. It is our hope that these and future studies using our DC EAE induction model will lead to therapies allowing the prevention or amelioration of multiple sclerosis.

Thus, our DC induction model of EAE allows the delivery of the encephalitogenic peptide Ac1–11 directly to the lymph node via migration of DC through the afferent lymphatics without the need for conventional adjuvants or in vitro T cell activation. Here, the DC encounter naive recirculating CD4 T cells and arrest their migration, allowing Ag-specific activation of MBP-specific T cells into capable EAE effector cells.

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References


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